Image processing-based automatic 3D quantification of sprouting angiogenesis in spheroid assays

Alvaro Jorge-Peñas[1], Marie-Mo Vaeyens[1], Arrate Muñoz-Barrutia[2], Carlos Ortiz-de-Solorzano[2], Peter Carmeliet[3], Maarten Roeffaers[4], Hans Van Oosterwyck[1,5]

[1]Biomechanics Section, Department of Mechanical Engineering, KU Leuven, Leuven, Belgium; [2]Cancer Imaging Laboratory, Center for Applied Medical Research, University of Navarra, Pamplona, Spain; [3]Laboratory of Angiogenesis and Neurovascular Link, Vesalius Research Center, KU Leuven, Leuven, Belgium; [4]Center for Surface Chemistry and Catalysis, KU Leuven, Leuven, Belgium, [5]Prometheus, div. Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium

Angiogenesis is the formation of new blood vessels from a preexisting vascular network. To date, several in vitro models have been developed to study sprouting angiogenesis from an assembly of endothelial cells that are embedded (endothelial spheroid assay and coated microcarrier bead) or seeded on the surface of a 3D matrix (invasion assay). Sprouting features are commonly evaluated from manual measurements, mostly from 2D projections of the acquired 3D z-stacks. However, manual analysis is time-consuming and prone to subjectivity and poor reproducibility. Therefore, there is a need for automatically quantifying sprouting capability in 3D to perform comparative studies among different experimental conditions. Here, we present the development of an image processing-based automated tool to extract a number of descriptors that quantify sprouting angiogenesis in spheroid assays, both in 2D and 3D.

Human Umbilical Vein Endothelial Cell (HUVEC) spheroids were embedded in a collagen type I hydrogel polymerized at 1.5mg/ml and cultured in endothelial cell growth medium-2 (EGM-2) for 24 hours at 37°C, 5% CO_2. Afterwards, the sprouts were fixed and stained with Alexa Fluor 488 conjugated phalloidin to be imaged with a laser scanning confocal microscope at 10x magnification.

The proposed method to analyze acquired images involves three steps. First, the main sprouting body, formed by the spheroid and connected sprouts, is thresholded using Otsu's binarization algorithm and separated from detached cells applying a connected-component labeling analysis. Second, a morphological opening operation with a spherical structuring element is applied to remove the spheroid. Finally, the end-points of the outer skeleton provide the locations at which touching sprouts are segmented.
We show how the segmented sprouts can be used to calculate descriptors of angiogenic features such as the number of sprouts and their length and orientation in the 3D space. Ultimately, this tool will allow faster sprouting analysis, providing higher objectivity and reproducibility.

The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ ERC Grant Agreement n° 308223)